

First record of Coliphage in Al-Qadisiya province water and using it as biomonitors of pollution

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Abstract :

Water pollution represent a certain challenge in the world , This work was carried from the period between March and April 2009 in attempt for screening one of the biological indicators which is a group of bacteriophages called coliphage. Obtained results showed that Al-Diwanyia river water contained about 78 ± 34 pfu/ml at the point of entering the city , while water samples which were collected from the center of the city contained a higher levels of coliphage about 234 ± 56 pfu/ml , but the highest levels of the phages 1023 ± 78 pfu/ml were documented at the last part of the river in the south of the city where the river leave the city .The laboratory investigation of coliphages ability to cause lyses to the bacteria explained that phage replication is correlated with the bacterial growth and increase with time, the complete lyses was recorded at about 8 hours. Study also included determination of soluble O_2 , electric conductivity., biological oxygen demand 5 , turbidity, temperature, and pH levels. Result explain that virological indicators could be a useful in biomonitoring of water quality.

Introduction :

Enteric viruses, such as Hepatitis A, Norwalk virus and Rotavirus are major agents of waterborne disease outbreaks such as gastroenteritis and hepatitis in humans.(1) Various bacterial indicator systems including coliform bacteria, the standard plate count, faecal streptococci and acid fast bacteria proved useful for assessing the biological quality and safety of a wide variety of waters. However, in view of the fundamental differences between bacteria and viruses, indicators more closely related to enteric viruses would be preferable for the evaluation of virological water quality (2, 3,4). Bacteriophages offer several advantages as models over virus detection in that they are non-pathogenic and methods for their recovery and enumeration are simple, rapid and inexpensive (1,5) . Coliphages are particularly useful for assessment of the safety of treated drinking water supplies because their absence has proven to be a reliable indication of acceptable virological and general hygienic quality (5,6). There are four main groups of bacteriophages that have been found to be useful for modeling virus transport/removal in the aquatic environment and treatment systems: coliphages (coliphages are phages which attack the bacterium *Escherichia coli*), F-RNA coliphages, *Bacteroides fragilis* phages and *Serratia marcescens* phages (1,2,5).It is known now that phages are useful means in environmental biotechnology in controlling sereous bacterial pathogens especially cholera and salmonella in water (6,7). Virological indicators of waters in Iraq still not used. This paper was done in an attempt to monitor the coliphages as representative for viruses levels in Iraqi water especially after the last epidemic out breaks of many bacterial and viral diseases such as cholera, hepatitis A, measles and typhoid fever and also to open the scope to control the danger water borne pathogens in Iraqi water especially cholera and salmonella.

Materials and methods:

Two experiments were carried one of them was aimed to monitor the phage levels in the river during it's passing through the city of Al-Diwanyia by choosing three stations the first one was at the north (when the river enter the city), second one at the

middle of the city and third one was at the south of city (when the river leaves the city) to evaluate the effect of waste disposal processes in the river along the city. This experiment was done by using the direct plaque assay according to (1,5,8,9,10). The second experiment was done to determine the pattern of phage replication, and the ability of phage to cause lyses to the bacteria, by using the specific host bacteria according to (11,12,13,14).

1- Water samples: samples were collected in clean containers about 500 ml, five replicates of samples were filtrated by using the milipore filter unit size 0.45µm, in an attempt to obtain phage containing water samples (15, 16).

2- Testing physiochemical parameters: parameters used were pH, E.C., BOD₅, turbidity, temperature and dissolved oxygen. Tests were carried directly and immediately for samples according to (17).

3-bacterial isolates: 18 hour broth of *Escherichia coli* isolate was obtained from (water) and diagnosed by microbiological techniques according to (18).

4-Phage assay: screening for coliphages was carried according to (9,10,18) as follows:
1. A 500 ml of sewage sample or water containing coliphage.

2. Dilute the sample 1 : 10 and 1 : 100 by making 10-fold dilutions in Tris buffer pH 7-7.2 by transferring 1.0 ml to 9ml of Tris buffer.

3. Melt four tubes of soft agar (0.7% agar/3 ml tube) by placing in a steambath or autoclaving. Place the agar in a water bath at 45–48°C and allow 15 min for the temperature of the agar to adjust to 45°C.

4. To the first tube add 1 ml of a log phase broth culture of *E. coli* 1 and 1 ml of undiluted sample. Remove the tube from the water bath and gently rock between your hands to mix the suspension for 2–3 seconds. Wipe the water from the tube with a paper towel and pour the agar over the Petri dish containing bottom agar. Quickly rotate the plate. Spread the top agar. Be sure the agar covers the entire surface.

5. Repeat Step 4 using 1 ml of bacteria and 1 ml of each phage dilution

6-Incubate for 24 hours at 37°C. Then calculate the plaques.

In the second experiment, 9 ml of 24 hours bacterial suspension containing 6×10^8 bacterium/ml was made by colony count and by photometric method in reference to McFarland comparators according to (15), was mixed with 1ml of 3×10^7 phage containing water and gently shaken, then incubated at 37°C at shaking water bath until complete clearing (lyses) finished then a lysates was monitored by plating the bacteria and phage each 2 hours (16).

Results and discussion:

Result obtained by this work represents the first documented in reference to the previous local environmental studies, which describe the levels of coliphage in Iraqi water. The results showed that the levels of phage increase as the river moves through the city which may be due to the increment of pollutants levels especially that cause the bacterial contamination in the river (1,5).

The results showed that the concentration of coliphages in river is about 78 ± 34 (plaque forming unit) pfu/ml (picture-1) which is less than this concentration at the second station 234 ± 56 pfu/ml, while, the higher levels were recorded at the last station which was chosen at the south of the city 1023 ± 78 pfu/ml as showed in (Fig-1). Previous studies monitored that the existence of the coliphages in fresh water is an indicator of the pollution with sewage water (17,19,20). Coliphages are valuable indicators of the presence of human enteric viruses in water because, in general, they outnumber viruses; they are at least as resistant to conditions in natural water environments and water treatment processes; and they are detectable by simple, inexpensive, and rapid techniques (5). The previous local environmental studies in

this area of this work had focused on bacteria, algae , invertebrates and chemical pollutants which indicated a remarkable elevated pollution in the river (21.22) . Chemo- physical investigation of water samples explained that the results were nearly similar to the results obtained by (19,20) from the same river except of BOD₅ values were elevated ,this elevation may be due to the acute decrement in the water quantity of the river which in turn reduce the dilution of pollutants and the unfortunately untreated or partially treated waste water disposal directly to the river . These results come in conformity with results obtained by many investigators (20) . The result of bacterial lyses was time correlated increment in phage titer which reach the maximal level in about 8 hours (Fig-2) by casing complete lyses (clearing) of bacteria in broth. This result was nearly similar to that obtained by previous investigators (15,16,20). The lyses results usually give a heterogeneous observations, the reason still unclear, but the presence of different phages types which replicate in *E. coli* may considered one of the cause, also it was documented that phage multiplication affected by physiological conditions such as bacterial physiological status and phage concentration in media, pH, temperature, salts concentration (14,15,18,20).

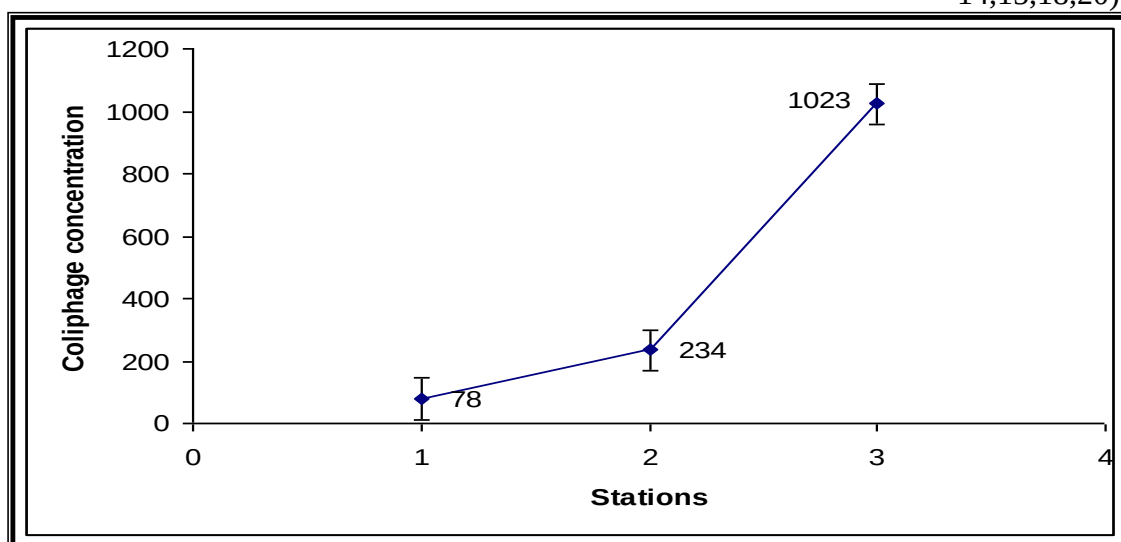


Figure -1: Levels of pfu in the river

Table 1: Chemo-physical parameters of river and heavy water

PARAMETER	STATIONS		
	1	2	3
pH	7.6±0.2	7.5±0.2	8.2±0.2
E.C.	1298±112	1401±123	1547±157
Dissolved O ₂	6.5±0.2	7.4±0.1	8.3±0.2
Turbidity	2.6±0.4	8.5±1.1	10±1.6
BOD ₅	1.5±0.1	1.6±0.1	1.8±0.3
Temperature	22.5	22.5	23.5

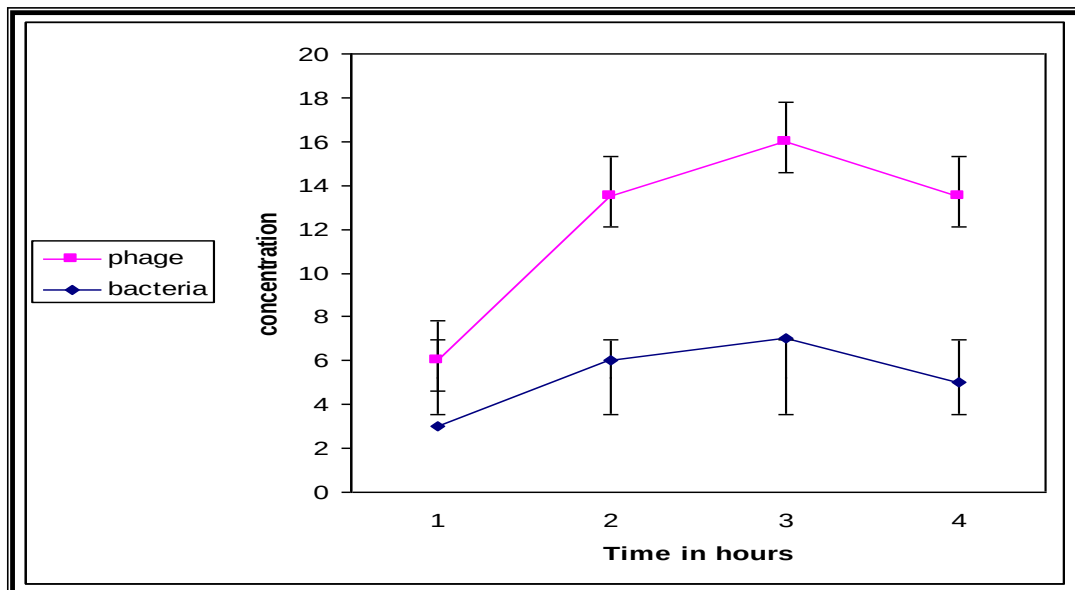


Figure-2: Growth curve of coliphages isolated from Al-Diwanyia river



Picture-1: Plaques of coliphages isolated from Al-Diwanyia river on culture of *E. Coli*

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